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Spectroscopic properties of various blood antigens/antibodies

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Abstract: Since the traditional method generates biological waste, there is a significant demand for an easy, quick technique of blood type identification without contamination. In fact, individuals can be divided into four main blood groups whose antigens are available in red blood cell (RBC) membranes and the antibodies in the plasma. Here, UV-vis and photoluminescence (PL) spectroscopic methods are systematically used to find the spectra of blood typing antigens (A, B and AB) and antibodies i.e. A-Anti, B-Anti, AB-Anti and D reagent. The PL spectra of RBCs in different blood groups as well as the corresponding antibodies are successfully resolved for the purpose of blood typing. The unique photophysical characteristics of these biomolecules including signal intensity and peak emission wavelength in PL spectra are lucidly anticipated to accurately discriminate ABO groups. PL spectra of RBC in positive blood typing indicate larger signal and shorter emission peak wavelength corresponding to negative ones. Furthermore, the monoclonal antibody PL emissions emphasize that Anti-A benefits higher intensity and shorter peak wavelength (blue shift) than B-Anti. In the following, lucid blue shifts are obtained in terms of antibody concentrations accompanying the elevation of fluorescence signal, most likely due to the aggregation induced emission (AIE) phenomenon, quite the opposite of the aggregation-caused quenching (ACQ) that is widely observed from conventional chromophore. Those are envisaged as unique properties of each antibody to utilize in the spectral blood typing.

1. Introduction

The ABO blood group is taken into account as most important blood typing system in human transfusion medicine being studied for more than a century. The first blood group system was discovered by Karl Landsteiner. The presence / absence of certain proteins, antigens, located on the surface of the red blood cells as well as the blood typing antibodies in the blood plasma lead to discriminate the blood groups (A, B, AB, O). In fact, those antibodies include Anti-B (in group A) and Anti-A (in group B) which mainly come from class immunoglobulin M (IgM) [1–4]. In fact, the amount of such blood typing antibodies in biological fluids is very low (IgM, 40–230 mg/dl), usually below the detection threshold of most of instruments [5]. The monoclonal Anti-A, Anti-B, Anti-AB reagents are known as blood typing antibodies of IgM class and D-Anti reagent originates from IgG class. D-Anti reagent is taken by chimeric human cell, however, the others are via mouse hybridoma. In the late 1950s, the chemical structures of those antibodies are investigated [6]. According to hybridoma technique, these reagents can be most likely replaced by human Anti-A and Anti-B typing in the commercial alternatives of such monoclonal [7].

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Several different methods have been extensively emerged to characterize the optical properties of biomolecules at cellular and subcellular levels. The non-invasive technique identifies the optical properties such as scattering, absorption and fluorescence properties of biomolecules [8,9]. Recently, photoluminescence (PL) and laser induced fluorescence (LIF) [10] are taken into account as the attractive alternatives for the biomedical applications [11–14]. For instance, these methods are known as a fast, low-cost, reagent free diagnostic tools that require low amount of fluid specimens during the analysis of the biological fluid components [15].

There are a number of articles reporting on immunoglobulins by making use of heating and UV exposure [16], studying antibody-ligand binding interactions and the binding constants [17], labeling of bovine IgG and goat IgG with Cy dye series [18], in line concentration measurements of IgG monomers and dimers [19], characterizing the modified IgG by singlet oxygen in methylene blue [20]. On the other hand, the behavior of blood typing antibodies in polar liquid solutions were carried out to obtain the THz properties of biological liquids [21]. The spectrophotometric method of human IgG was performed in a wide concentration range [22]. A method for the mid-infrared spectral quantification of IgG has been developed in horse blood plasma [23]. The pre-processed SERS spectra is also examined in order to evaluate the spectral differences among ABO blood groups and the antigen- antibody interactions [24].

On the other hand, the transferase enzyme is responsible for the addition of specific sugar to an oligosaccharide precursor chain having a terminal galactose. Figure 1(a) illustrates structure of the blood type O antigen attached to RBC membrane that is formed by affecting fucosyltransferase to add a fucose sugar in the terminal galactose. A and B antigens are formed by adding N-acetylgalactosamine and galactose to the terminal O-antigen respectively located on the RBC membranes as shown in Fig. 1(b) and 1(c). Note that Porphyrin as part of hemoglobin in RBC is taken into account as the major fluorescence component [25]. Moreover, the Rh antigens is a part of a protein complex in the RBC membrane. The Rh positive and Rh negative refer to the Rh antigen's presence/absence. Unlike most of superficial cell molecules, Rh proteins are not oligosaccharides, that are made up of 417 amino acids distributed over six extracellular segments.

On the other hand, Fig. 1(d) depicts the prototype class of a typical antibody. The basic unit of an antibody molecule consists of four polypeptides in the form of a couple of heavy and light chains. It is Y-shaped, with a couple of identical antigen-binding sites at the ends of the arm structure. Each polypeptide chain includes a constant variable region (VH, VL) that exhibits great variability in amino acid sequence. The greatest variability in the light chain is called hypervariable regions or complementarity-determining regions (CDRs) which are located around residues at 30, 55, and 95 amino acid sequence characterizing the specificity of the antibody [26].

The analysis of sequence database emphasizes that antibodies contain plenty of amino acid residues located at head (CDRs) [27]. The degree of antibody specifity and affinity in combining site are attributed to the abundance and content of the amino acid residues. Furthermore, concentration, sequence, stereochemical and electronic features of amino acids in light and heavy chains determine antibody specificity in the CDRs. In fact, the diverse composition percentage of amino acid residues in different parts of antibody structure alter the shape and polarity to interact with structurally diverse antigens as variety of antibody specificity. Particularly, the interactions between positive charge groups and significant aromatic ring residues i.e. tryptophan (Trp), tyrosine (Tyr) and phenylalanine (Phe) are highlighted. The aromatic ring residues exhibit strong fluorescence properties that obviously change the fluorescence spectra of various antibodies.

The comparative sequence analysis of CDRs of human antibodies via reverse transcription polymerase chain reaction certainly attests the differences in the amino acid sequences of variable regions. Particularly, CDR3 in the VH or the VL regions distinguish the antigen structures [28]. In fact, those recognize different carbohydrate antigens given by the sugars with a terminal galactose for Anti-B or a terminal N-acetylglucosamine in Anti-A.

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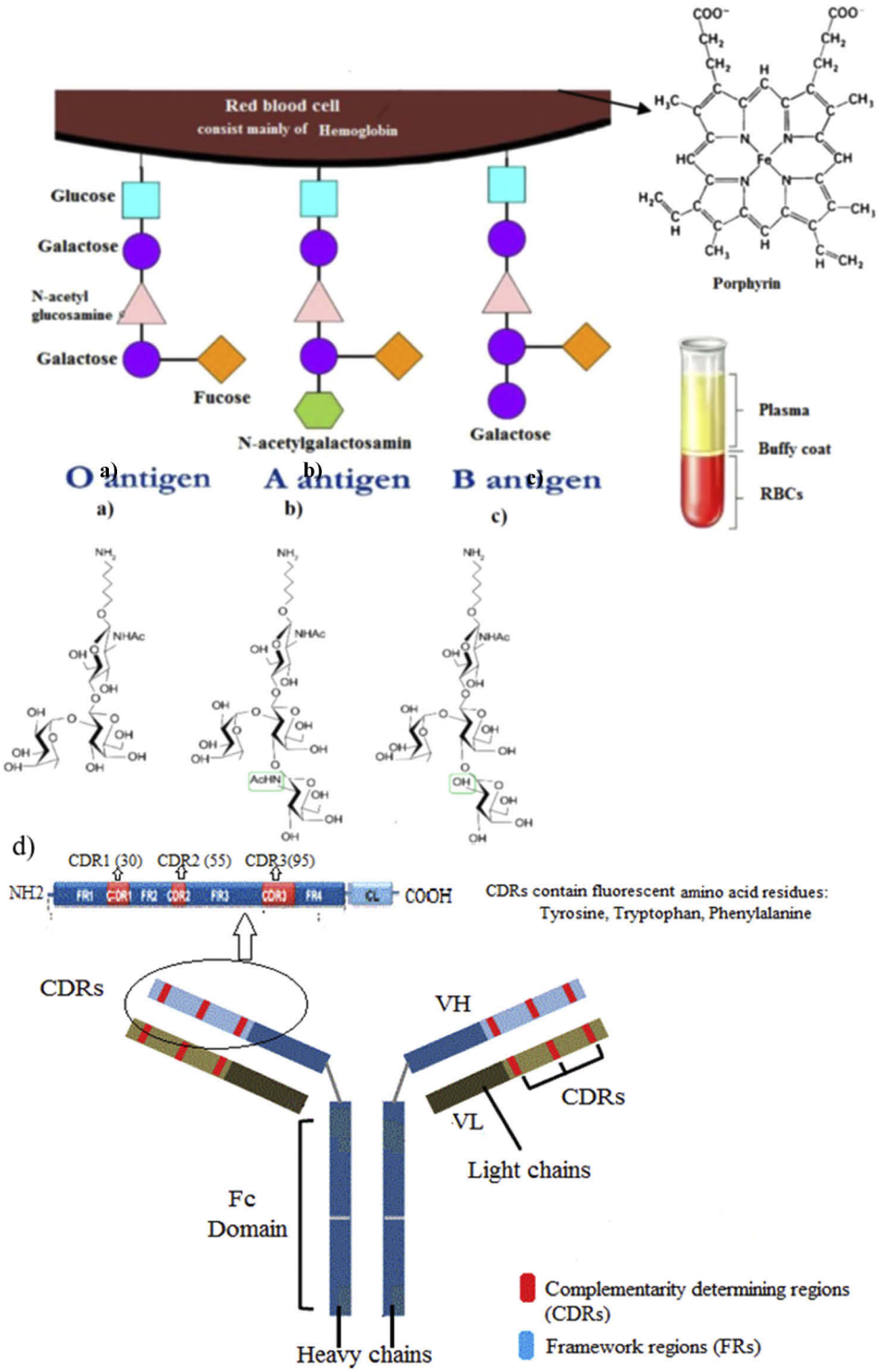

Fig. 1. Chemical structure of various blood antigens and antibody. a) O antigen b) A antigen c) B antigen d) Schematic of a typical antibody containing FC and Fab domains including CDRs and FRs in heavy (VH) and light (VL) chains. Note that amino acid ring residues such as Trp, Tyr and Phe are sequentially located in CDRs. Figure 1 right illustrates the components of whole blood after centrifuging.

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In fact, a number of parameters deals with the molecular structures and the surrounding environment to affect the fluorescence quantum yield and the corresponding spectral shift of an organic biomaterial. The aromatic compounds, in which first excited singlet states (S_1) is a conjugated (π, π^*) system, tend to a higher fluorescence quantum yield. Aromatic single-rings are characterized for the fluorescence emission in the ultraviolet region and the aromatic multi-rings are featured over the visible region. The electron - donating groups such as - OH, -NH_2 and -NR_2 enhance the fluorescence efficiency, whereas electron - withdrawing groups such as -CHO, -CO_2H and -NO_2 reduce the fluorescence quantum yield. In a non-polar environment, the emission of fluorophores naturally shifts to shorter wavelength (blue shift). Conversely, the peak of emission moves to a longer wavelength (red shift) in the polar environment [29,30]. Table 1 tabulates the different chemo-physical parameters involving the variation of signal intensity and emission wavelength.

Table 1. The main parameters for fluorescence signal reduction/enhancement and red/blue shift.

Fluorescence signal enhancement	Fluorescence signal Reduction	Red shift	Blue shift
i) The electron donor groups such as - OH, -NH_2 and -NR_2	i) The electron acceptor groups such as -CHO, -CO_2H and -NO_2	i) Polar environment such as carbonyl, hydroxyl and oxygenate groups.	i) Non-polar environment such as Lipophilic media
ii) Aromatic compounds (conjugated π- π system)	ii) ACQ	ii) Reabsorption	ii) AIE
iii) AIE			iii) ACQ

In clinical standard method, the blood typing is categorized as the forward and reverse ones. In forward typing, the blood sample is mixed with monoclonal antibodies (A and B antibodies). Then, the sample is checked whether any agglutination takes place. If the blood cells were agglutinated together, then those would react with the antibodies of the same label. On the other hand, the reverse blood typing refers to the plasma examination containing ABO antibodies. Drops of plasma is mixed with the known RBCs i.e. A / B types, and the agglutination patterns are observed to identify blood type.

To the best of our knowledge, this work is carried out for the first time on the context of blood antigens/antibodies based on UV-Vis and PL spectra assessment. By making use of spectral absorbance, the proper absorption line is selected to excite the reagents of interest to obtain PL spectra. A vigorous attempt has been made here for the preparation, detection and differentiation of the various spectral blood antibodies/antigens. This approach intends to develop an alternative blood typing method without imposing any biological contamination.

2. Materials and methods

Blood mainly consists of erythrocytes (RBCs), leukocytes (WBC) and fluid plasma including plenty of proteins and biomacromolecules such as globulins, glucose, albumins, hormones, cholesterol and etc. At first, the preparation process of antigens is carried out. The large number of donors would give confidence that the results generalize well and are not due to any possible confounding factors between individual donors. For this purpose, whole blood is taken from healthy volunteers of type $A^{\pm}$, $B^{\pm}$, $AB^{\pm}$ and $O^{\pm}$. Then, the blood sample per volunteer is injected into vial for centrifuging at 3000 rpm in 5 minutes. According to Fig. 1(right), the plasma and buffy coat layers are separated such that those components would appear above RBCs. Subsequently, the proteins in plasma and existing antibodies are washed out from vial content as to RBCs remain for further purification. Then, RBCs are diluted by saline (0.9% NaCl) and centrifuging is repeated again. Afterwards, the supernatant is removed and the process is repeated for several times as long as its color changes from yellowish to transparent to give out

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a pure RBCs suspension plus certain antigens. Note that the blood typing antigens are naturally attached to RBCs membrane. Now, RBCs are ready for the next analyses. This process has been carried out in Tehran Blood Transfusion center for each blood sample taken per volunteer. On the other hand, according to our demand, the natural blood group antibodies are supplied by CinnaGen where hybridomas between human and mouse myeloma cells are used to generate antibodies based on the common procedure. The normal saline dilutes the samples of interest.

Furthermore, the absorbance spectra of the blood antibodies are recorded using Jenway 6715 UV-Vis spectrophotometer with a pulsed Xenon light source scanning the spectral range over 190 nm - 1100 nm. It is equipped with dual silicon photodiode detector coupled with the spectrometer having 1.5 nm resolution. For the irradiation of sample, a quartz cubic cuvette (1 cm $\times$ 1 cm $\times$ 2 cm) is employed. Moreover, Perkin Elmer luminescence PE 45, with spectral resolution of 1 nm, is exploited for the PL measurements. This utilizes a coherent pulsed Xenon flash lamp ranging 200-1100 nm equipped with photomultiplier array detector.

In the case of RBCs (antigens), around forty specimens were examined in general. Different volunteers per type were selected and the process was repeated in favor of eight blood type where each group contains 5 volunteers. After clinical standard blood typing, then the optical specta were recorded and statistical analyses were carried out accordingly. On the other hand, in the case of antibody tests, around ten samples per type were examined. The mean value and SD of each data point are assessed. The PL signals and peak emission wavelengths show statistical significance (P-value$<$ 0.05) such that one can obviously discriminate the spectra of various blood types. It is worth noting that SD mostly arises from instrumental errors addressing the peak to peak output stability fluctuation of Xe flash lamp in spectrometers to be less than 3%. Hence, the fluctuation in PL spectra of each sample during repetitive trials would be less than this value. Furthermore, spectral shift error is negligible ($\sim$1A$^\circ$) to be much smaller than the resolution of spectrometers (UV-visible 1.5 nm and PL 1nm) so not to be detected by these instruments. In fact, other sources of error are due to the statistical populations and the concentrations under test.

3. Results and discussion

Specific sugars attached to the precursor substance on the red cell membrane determine ABO antigenic activity. In O and A antigens, there are two monomers of galactose (Gal, $C_6H_{12}O_6$), whereas B antigens contain three monomers of galactose on the membrane. An amino sugar derivative of galactose, N-Acetylgalactosamine (GalNAc, $C_8H_{15}NO_6$), in terminal carbohydrate forms the antigen of blood group A. On the other hand, the Rh positive and Rh negative refer to the attendance /absence of the RhD protein that encodes the Rh antigen. The absence of the antigen, especially Rh complex, alters the RBC shape. The alteration in molecular shape changes the energy states leading to vary the fluorescent properties. Furthermore, by monitoring molecular fluorescence characteristics, some knowledge can be understood regarding the nature of those macromolecules.

Here, we intend to find intrinsic fluorescence of RBC in normal saline solutions. Literature attests that the hemoglobin in RBC benefits auto fluorescent property [25]. In the hemoglobin molecule, the Porphyrin consists of four pyrrole rings (tetrapyrol) responsible for absorption and emission in the visible region [31]. At first, antigen solutions are provoked at 410 nm. Fig. 2(a) depicts the PL emission spectra of different blood typing RBCs. Figure 2(b) represents the spectral absorbance of Porphyrin and the corresponding fluorescence emission spectrum emphasizing an absorbance peak wavelength λ_p at 410nm, following an intense fluorescence emission at 660nm over a wide spectral range of 500 nm - 750nm [31]. This reveals the dominant effect of Porphyrin in RBC fluorescence emission. Figure 2(c) and 2(d) illustrate the emission intensity and the corresponding peak emission wavelength of various RBCs with blood typing antigens attached at their membrane respectively. In comparison, O^+ indicates larger fluorescence signal against that of O^- (RBC without any antigens) due to the contribution of Rh antigen

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attached to O^+. Moreover, the latter shows a blue shift of 1.5 nm with respect to that of O^-. In fact, the presence of non-glycosylated Rh antigen with hydrophobic profile not only changes the shape of the RBC but also reduces the polarity of environment leading to a measurable blue shift (for instance, A^+ has a blue shift ~2nm with respect to A^-). Moreover, the Rh antigen conjugation in RBCs usually creates such a blue shift. In addition, carbohydrate ABO antigens contain carbonyl, hydroxyl and oxygenate groups with higher degree of polarity leading to a certain red shift as shown in Fig. 2(d). On the other hand, the presence of OH and NH_2 groups in ABO antigen structures elevate the intensity of fluorescence signal [29,30] according to Fig. 2(c) and Table 1. The maximum fluorescence signal takes place in RBCs of type AB^+. The largest red shift is attributed to AB^- blood typing. The difference in RBC fluorescence signals in A and B types usually arise from the presence of OH and AcHN groups. Eventually, we come to conclusion that a lucid red shift takes place for most of antigens originating from the specific sugar chains attached to RBC surface. This demonstrates a unique fluorescence characteristic for each antigen utilizing Porphyrin as the base fluorophore in the RBC's structure. As a consequence, ABO group can be well differentiated using PL spectra.

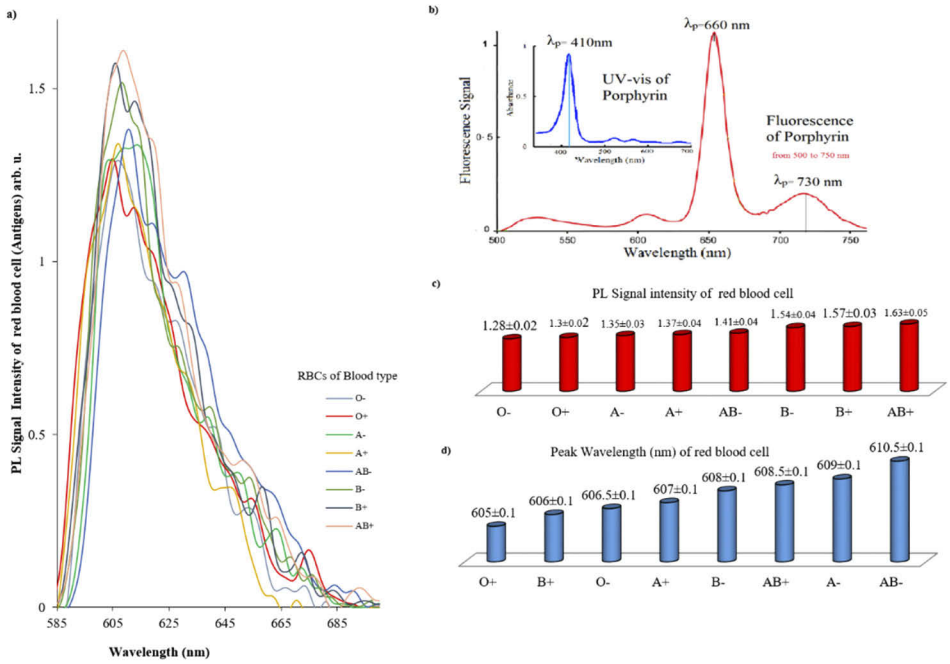

Fig. 2. (a) PL spectra of the red blood cell (including hemoglobin and antigen) at λ_{ex}=410 nm. (b) Spectral absorbance of Porphyrin with an absorbance wavelength peak of λex=410 nm and the corresponding fluorescence spectrum from 500 to 750 nm [31] (c) PL signal and (d) Peak emission wavelength for various red blood cells.

Figure 3(a) illustrates UV-vis spectral absorbance of the various blood typing antibodies. UV-vis spectra contain three absorption peaks that lie at UV and visible regions. Those absorbance peaks take place mainly around 260nm, 420nm and 560nm. The spectral absorbance drastically differs according to their unique molecular transitions. Figure 3(b) illustrates the spectral absorbance of significant amino acid residues i.e. Trp, Tyr and Phe at the UV spectral region [29]. Those residues are sequentially located in CDRs of VH and VL. A strong band at 210–220 nm and another band at 260–280 nm appear for Trp (280nm), Tyr (274nm) and Phe (257nm). Figure 3(c) depicts the absorbance amplitudes of antibodies of interest. Figure 3(d) demonstrates the absorbance peak wavelength at 248.83 nm, 249.8 nm, 255 nm and 260nm

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for D reagent, A-Anti, AB-Anti and B-Anti respectively. It is supposed that major aromatic ring residues i.e. Trp, Tyr and Phe are responsible for the absorbance/fluorescence properties of antibodies. The discrepancy is most likely due to different amino acid sequence of variable regions and divers length in sequence such that spectrally shift the absorbance peak to shorter wavelength, respect to Trp absorbance. According to UV-vis spectra, 260 nm wavelength is selected as excitation wavelength to obtain PL spectra.

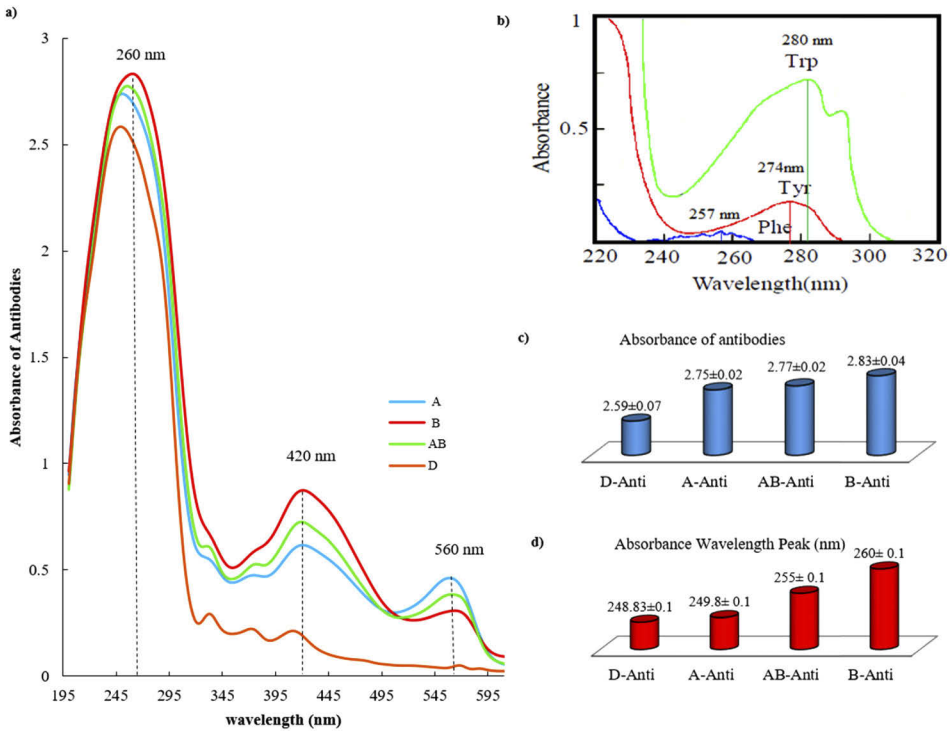

Fig. 3. (a) UV-Vis absorbance spectra of the various blood typing antibodies, dominant peak takes place at UV region. (b) Absorbance spectra of Trp, Tyr and Phe amino acid residues [29] (c) Absorbance intensity around 260 nm for antibodies (d) Corresponding peak wavelength of antibodies. Note that these data are unique and reproducible as the main features of each antibody.

Figure 4(a) illustrates the PL spectra of A-Anti, B-Anti, AB-Anti and D reagent respectively. One believe that aromatic ring residues are responsible for the fluorescence properties of proteins in the UV spectral region. Figure 4(b) depicts the fluorescence intensity of significant amino acid residues. The characteristic fluorescence peak takes place almost at 350 nm, 300 nm and 280 nm for Trp, Tyr and Phe respectively [29]. Figure 4(c) shows PL emission signal of the antibodies. Figure 4(d) displays corresponding peak emission wavelengths at the 343 nm, 343.5 nm, 344.5 nm and 345.5nm of A-Anti, D reagent, AB-Anti and B-Anti respectively.

The CDRs and FR regions of ABO antibodies possess high homology with 53-100%, however the sequence of amino acids in CDRs display identities of 35-78% with diverse length in sequence [32]. That is why the intrinsic fluorescent characteristics of natural antibodies i.e. Anti-A, Anti-B, Anti-AB find similar profile accompanying distinct spectral shifts. The fluorescence profiles mainly arise from the individual aromatic residues. Despite Tyr exhibits more intense fluorescent emission than Trp in solution, however here Trp looks like the dominant peak around 350 nm. In general, physical and chemical structures affect on the peak emission wavelength. The correlation

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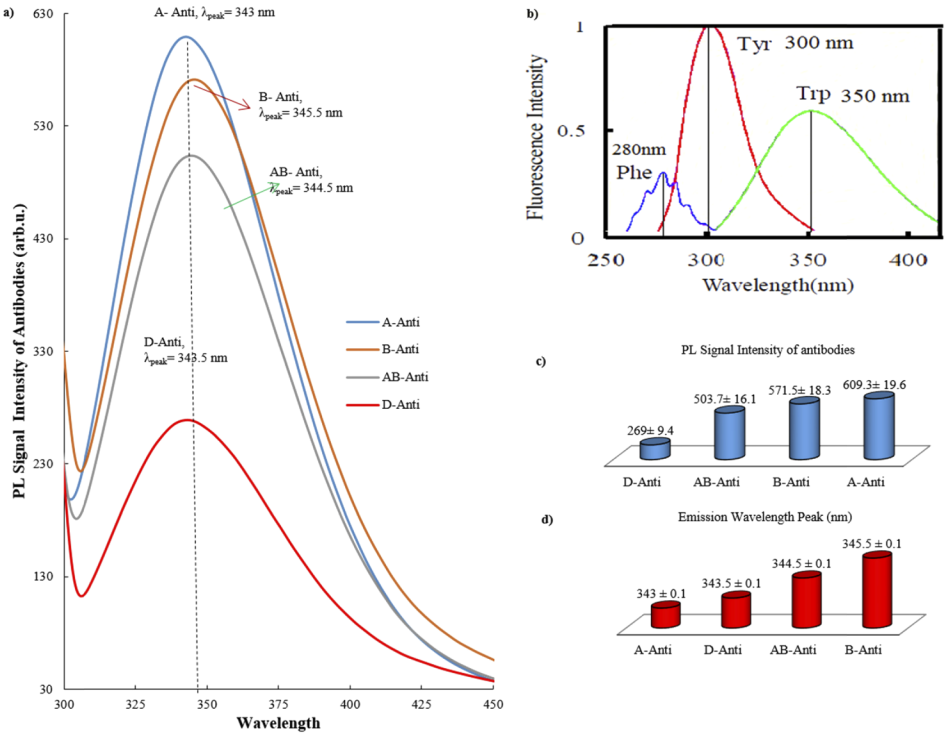

Fig. 4. (a) PL spectra of various blood typing antibodies at $\lambda_{ex} = 260$ nm. (b) Fluorescence spectra of Trp, Tyr and Phe amino acids residues [29] (c) PL emission intensity (d) Corresponding peak wavelength of antibodies. Note that the Trp fluorescence emission resembles to be dominant due to presence of fluorescence peaks around 343-345.5 nm.

of spectral shift in antibodies with aromatic ring residues in CDRs and the neighborhood is not well understood. This may be modeled via molecular dynamic and density function theory (DFT) via typically Gaussian software package.

Figures 5(a) and (b) display the PL emission spectra in terms of the concentrations for A-Anti and B-Anti respectively. Figures 6(a) and (b) illustrate the PL emission spectra in terms of the concentrations for AB-Anti and D reagent respectively. The relative signal amplitudes (up inset) and the corresponding spectral shifts (down inset) vary in terms of monoclonal antibody concentrations (1, 2.5, 5, 7.5 and 10 mg/ml). Regarding data fitting, the exponential and lorentz equations are used to fit the data for the up and down insets respectively according to OriginPro2015 software. Furthermore, R^2 goodness values are obtained based on LSM fitting and inserted into legends. The larger R^2 indicates better model of data fitting.

According to signal amplitudes and corresponding spectral shifts in Figs. 5 and 6, it resembles that the contribution of Trp in PL spectra is dominated. According to the Lambert-Beer law when a monochromatic light beam propagates through a sample, the optical energy can be much absorbed at dense concentrations. Here, PL emissions undergo a nonlinear elevation in terms of the concentration. The wavelengths of PL emissions experience a sharp rise at dilute solutions and then follow a smooth decay at dense concentrations. The elevated signal mainly arises from the larger population of the aromatic residues of amino acids. At first, bathochromic effect (red shift) of emission wavelength takes place as a consequence of low concentrations. Then the molecules are sterically congested, where the hypsochromic effect (blue shift) becomes dominant [33,34]. Consequently, the blue shift (down inset in Figs. 5(a), (b), c, d) is due to the weak

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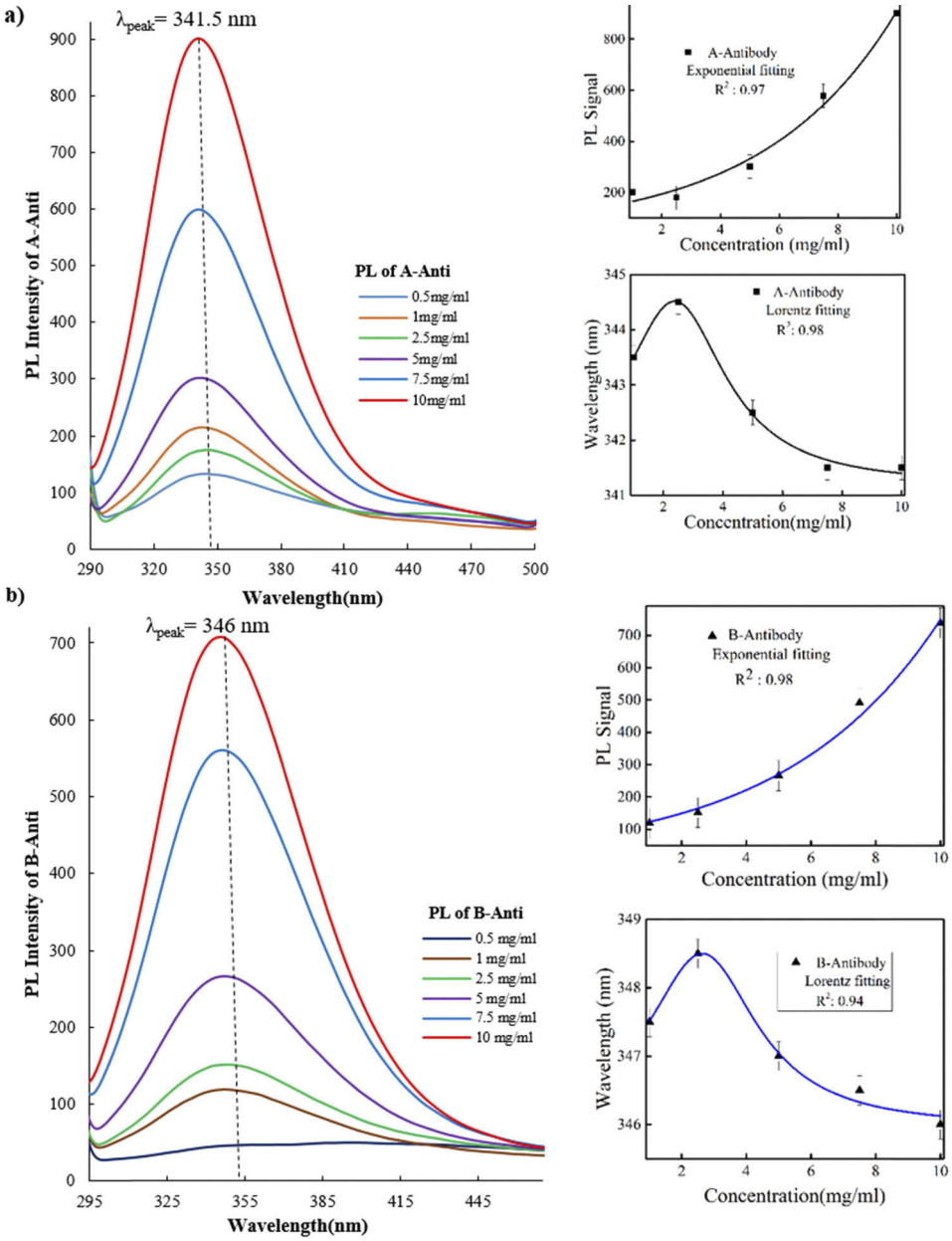

Fig. 5. The PL signal intensity in terms of concentrations (1, 2.5, 5, 7.5 and 10 mg/ml). (a) A-Anti (b) B-Anti. Signal amplitudes and spectral shift versus each antibodies concentration are depicted in up inset and down inset graphs respectively.

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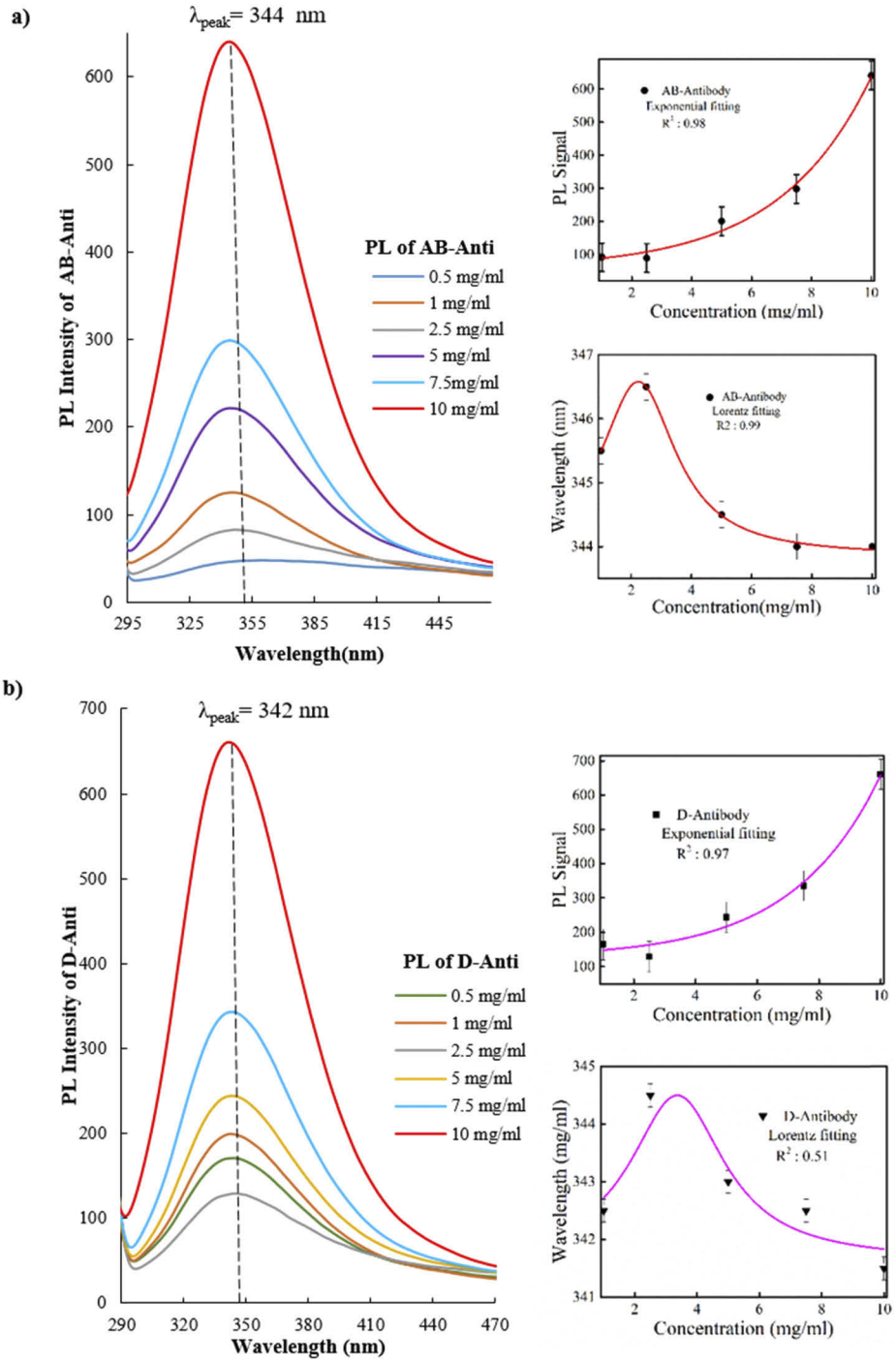

Fig. 6. The PL signal intensity in terms of concentrations (1, 2.5, 5, 7.5 and 10 mg/ml). (a) AB-Anti (b) D-Anti reagent. Signal amplitudes and spectral shift versus each antibodies concentration are depicted in up inset and down inset graphs respectively.

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intermolecular interactions. The latter resembles to be weaker than solute-solvent interactions because the intermolecular interactions are well prevented due to the bulky structure of the molecules. The mechanism is quite different from our previous works [8,14]. In general, the static self-quenching of fluorophores are dominant accompanying a blue shift and simultaneously the fluorescence signal reduces with dye concentration due to the aggregation-caused quenching

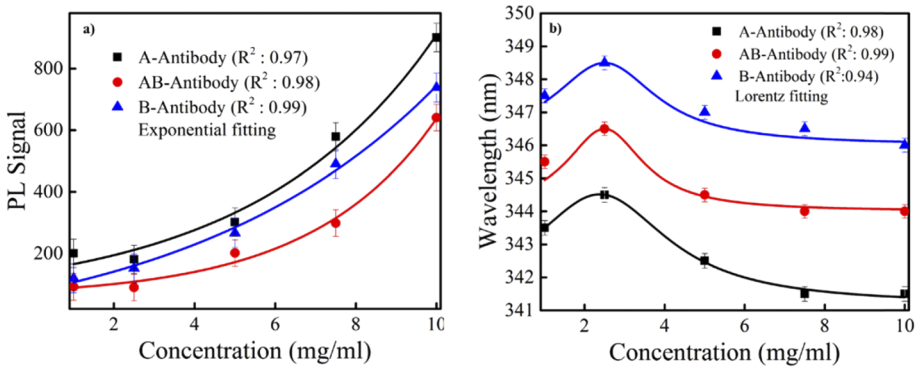

Fig. 7. (a) PL signal and (b) peak wavelength of A-Anti, B-Anti and AB-Anti versus concentrations (1, 2.5, 5, 7.5 and 10 mg/ml) in saline solution.

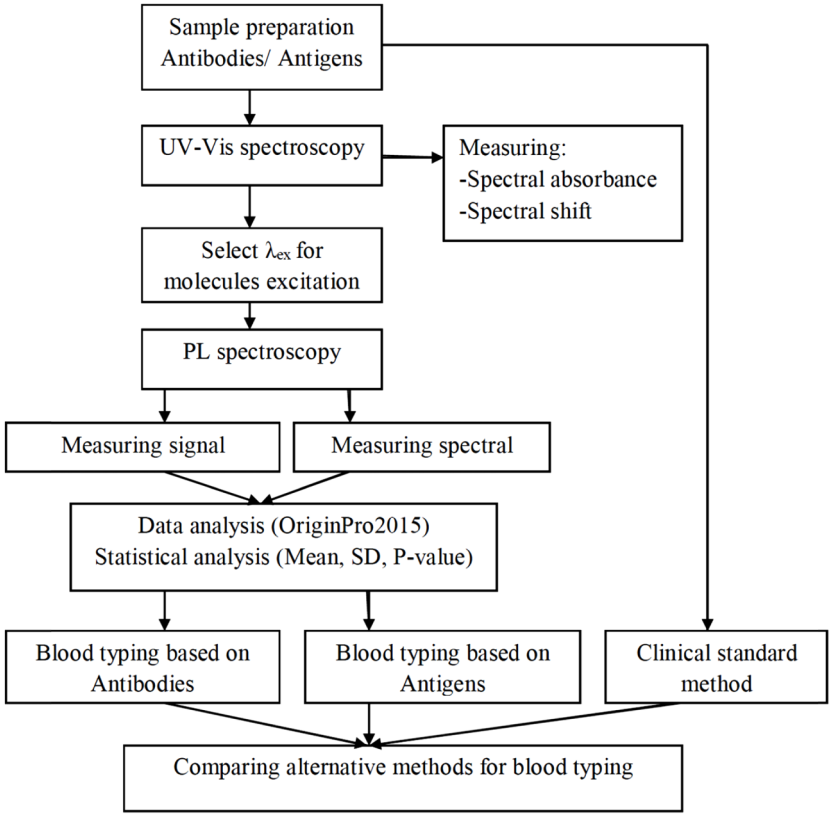

Fig. 8. Block diagram of blood typing based on spectrophotometric approach and traditional clinical method.

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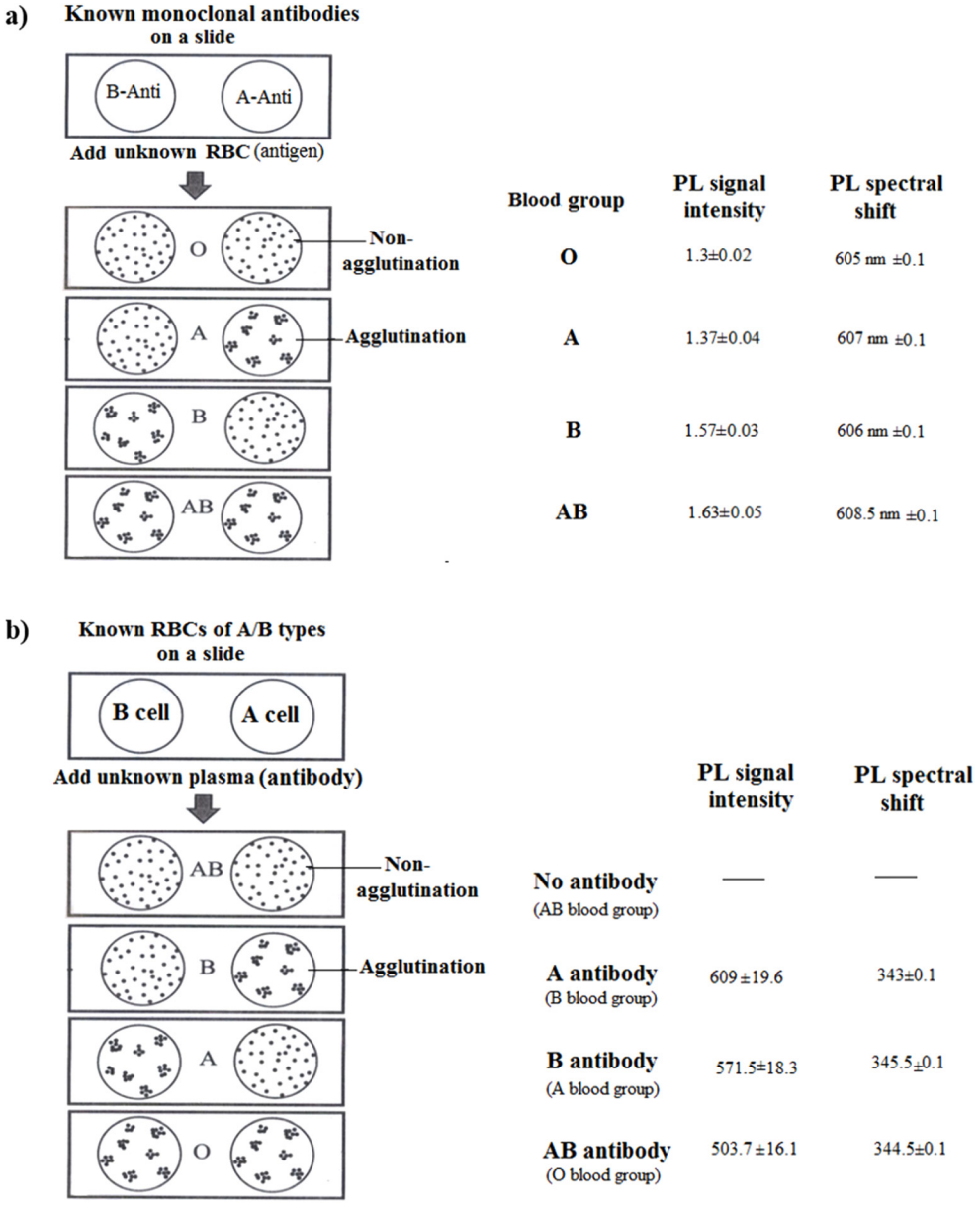

Fig. 9. Common clinical procedure a) forward b) reverse blood typig versus PL spectroscopy of antigens and antibodies as alternative methods of blood typing. Note that AB blood type contain no antibodies, therefore no signal is detected.

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(ACQ). Here, the initial red shift at dilute solution arises from reabsorption events, accompanying fluorescence signal deduction. In following, the blue shift is associated with signal elevation mainly due to the aggregation induced emission (AIE). This process is the direct opposite of ACQ, which is well-known and widely observed from conventional chromophores.[33–35], In fact, most organic compounds benefit planar structures with high photoemission in solution. However, some organic luminophores have freely- rotating groups with high rotational degree of freedom. When those molecules are excited instead of releasing that energy as light, they would relax back down through those rotational states. However, in the course of aggregation, those luminophores most likely would restrict the rotational pathways. This allow those molecules to become very emissive leading to increase the photoluminescence via radiative processes and subsequently larger quantum yield is obtained. Therefore, the non-planar molecules bearing bulky peripheral groups can constitute amorphous-like aggregates with significantly weakened intermolecular interactions, and thus, can promote AIE property. The blood typing antibodies are taken into account as the nonplanar biomolecules such that the aggregation blocks the non-radiative pathway to enhance the photo emissions via radiative decay. In general, Fig. 7(a) and Fig. 7(b) depict the PL signal and the peak wavelength of A-Anti, B-Anti and AB-Anti versus concentration in saline solution respectively. Those are envisaged as distinct and unique property of each antibody that can be employed for the spectral blood typing in future.

In summary, for convenience, Fig. 8 demonstrates the sequential steps of blood typing based on spectrophotometric antigen/antibody approach that has been extensively carried out in this work. The spectral findings are compared to the traditional method.

Figure 9(a) illustrates the schemes of forward and fig. 9(b) displays reverse traditional blood typing methods versus PL spectroscopy as the antigen/antibody alternative assessment. In clinical standard forward typing method, monoclonal antibody drops on a slide and the un known RBCs are added to them to find out agglutination/ non agglutination patterns. The reverse blood typing refers to the plasma examination containing ABO antibodies. This method relies on known RBC drops on a slide whose mixture with unknown plasma give out agglutination/ non agglutination patterns.

Eventually, nominal processing time for the optical method is ~ 12 min against that of traditional blood typing to be ~15 min. However, the preparation time for RBC purification can be automated to shorten the required time less than 8 min relying on PL spectroscopy in lieu of traditional technique. Bio-hazardous agents such as cultured animal cells and the products taken from lab animals certainly burden additional dangerous infections. Since monoclonal antibodies are usually used for blood typing in traditional method, and those are obtained from mouse hybridomas therefore it is prone to the biological pollution of microorganisms. Those are taken into account as potential superiority of the proposed technique against the traditional one.

4. Conclusion

This work deals with analysis of blood antigens (A-Antigen, B-Antigen and AB-Antigen) and blood typing antibodies by making use of UV-Vis and PL spectra which is a continuation of our previous works on the investigation of the fluorescence properties of bio-molecules. Here, the PL spectra are obtained at the excitation line of 400nm for RBCs containing carbohydrate antigens. Similarly, protein blood typing antibodies are provoked at 260 nm to assess their photoluminescence features. It is demonstrated that ABO antigens at RBC membranes as well as A- Anti, B-Anti, AB-Anti and D reagent are well differentiated by virtue of spectral fluorescence emission. Additionally, the organic compounds of interest exhibit unique fluorescence behavior in terms of concentrations.

The RBCs PL spectra of positive blood typing attest higher signal intensity and shorter emission peak wavelength respect to the negative ones. In fact, the fluorescence spectra of antigens arise from that of Porphyrin as main part of hemoglobin and other related compounds. On the other

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hand, discrepancy in PL emissions of antibodies delineates that A-Anti gains larger signal and shorter peak wavelength than that of B-Anti. The spectral signatures are revealed for different types of antibodies whose main luminophores are the aromatic amino acids i.e. Trp, Tyr and Phe located in CDRs. Since the PL spectra of antibodies are most likely similar to Trp, hence, UV-vis and PL manifestations of this amino acid is taken into account as a characteristic feature. However, Tyr and Phe aromatic ring amino acid in CDRs play a secondary role. Furthermore, the relative signal intensities and the spectral shifts in PL emissions are measured in terms of antibody concentrations. PL signal nonlinearly elevates versus antibody concentration, whereas a red shift occurs at dilute solutions and then a lucid blue shift takes place at dense suspensions. The PL signals and corresponding spectral shifts versus concentration exhibit unique features of antibodies that are vividly realized in the ascending order from A-Anti, AB-Anti to B-Anti unlike conventional fluorophores which obey ACQ and just in agreement with AIE phenomenon of photoemission. Here, the nonlinear PL signal elevation and corresponding blue shift appear versus concentration to reveal AIE phenomenon in blood typing antibodies.

As a consequence, not only the fluorescence spectroscopy of antigens/antibodies can be employed for identifying different types of blood, but also introduces a non-invasive and reagent free diagnostic tool for blood typing. Finally, Table 2 tabulates the abbreviations that are given throughout the text and corresponding explanations.

Table 2. The explanation of abbreviations.

RBC	Red blood cell	Trp	Tryptophan
WBC	White blood cell	Phe	Phenylalanine
ABO	Blood typing antigens (A, B and AB)	PL	Photoluminescence
VH	Variable domains in the heavy chain	AIE	Aggregation induced emission
VL	Variable domains in the light chain	ACQ	Aggregation-caused quenching
CDRs	Complementarity determining regions	DFT	Density function theory
Tyr	Tyrosine	UV	Ultraviolet

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Disclosures

The authors declare that there are no conflicts of interest related to this article.

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